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Phlebotomine sand flies (Diptera, Psychodidae) from iron ore caves in the State of Pará, Brazil

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Abstract

The present study aimed to evaluate the distribution of sand fly species in iron ore caves in the State of Pará, Brazil and to associate the richness and abundance of these insects with the capacity of leishmaniasis transmission. Entomological captures were carried out in the years 2010, 2013, 2014 and 2015, throughout active samples with brushes, along the entire caves' extension, in dry and rainy periods. A total of 9,807 sand flies were counted during the 532 samplings events, being 4,340 in the dry period and 5,467 in the rainy period. A random sample of 802 morphologically identified specimens consisted of 8 genera and 17 species, being 369 males (46%) and 433 females (54%). The predominant species was *Sciopemyia sordellii* with 60.6% of the total of sand flies collected. Differences in composition and richness were observed between caves located inside of forest and anthropized areas. The mean richness and abundance were different between the wet and rainy periods, with a greater abundance of these insects in the rainy period. The phlebotomine fauna proved to be rich and abundant in the sampled caves, however, environmental degradation seems to be the main factor determining changes in the composition and richness, reinforces the importance of these places as a shelter for sand flies in degraded areas.

Keywords

Leishmaniasis, Phlebotominae, Vector ecology

Introduction

Phlebotomines (Diptera, Psychodidae, Phlebotominae) are insects with medical importance because include vector species of *Leishmania* sp., *Bartonella bacilliformis* and arbovirus (Alvar 2001; Sherlock 2003; Lainson and Shaw 2005; Kamhawi 2006; Battisti et al. 2015). They usually take shelter in places with high moisture content and with organic matter available, shaded areas and drafts to prevent desiccation (Aguiar and Medeiros 2003).

Caves are favorable environments for the occurrence of these insects due to the stable conditions of temperature and humidity (Freitas and Littlejohn 1987; Freitas 2010; Lauritzen 2018). Vertebrates such as bats, rodents, birds, lizards and amphibians can serve as a blood source for sand flies present inside and around cavities (Auler et al. 2019).

In Brazil, the data regarding the phlebotomine fauna in caves are incomplete (Galati et al. 2003; Galati et al. 2006; Alves 2007; Barata et al. 2008; Galati 2008; Almeida et al. 2019; Campos et al. 2020) several species of sand flies have been described (Alves et al. 2008; Carvalho et al. 2010, 2011; Barata et al. 2012; Vilela et al. 2015).

In the Serra do Carajás, located in the state of Pará, the mining potential of the Curionópolis and Parauapebas municipalities has attracted enterprises that, despite contributing to the economic development of the region, have caused major environmental changes, with direct impacts on biodiversity and public health (Palheta et al. 2017). Since the 1980s, several territorial, economic, social and political conflicts have emerged or were intensified by the mining activities, such as the emergence of municipalities, population growth and increased environmental pressures on land use (Palheta et al. 2017).

Serra de Carajás has a large number of caves with economic and biological potential, but it is also considered an endemic area for cutaneous leishmaniasis (Ward et al. 1973). The expansion of municipalities and their peripheral populations living close to the caves may represent a risk of *Leishmania* transmission. In this sense, the present study aimed to evaluate the distribution of phlebotomine species in the caves and to associate the richness and abundance of these insects with the potential transmission of leishmaniasis. In addition, we sought to contribute to the knowledge of the region's biodiversity, given the economic and biological potential of the iron ore caves of the State of Pará.

Methods

Study area

The study was carried out in iron ore caves located in the regions of Morro I, Morro II (Serra Norte), within the Flona de Carajás and Serra Leste, outside a conservation unit, in the municipalities of Parauapebas and Curionópolis (Figure 1). In Parauapebas, the altitude of the caves varied from 350 to 650m asl and in Curionópolis it varied from 230 to 560m asl. Parauapebas (6°04'03"S, 49°54'08"W) is a Brazilian municipality located in the state of Pará, northern Brazil. Curionópolis (6°3'58"S, 49°33'40"W) is located around

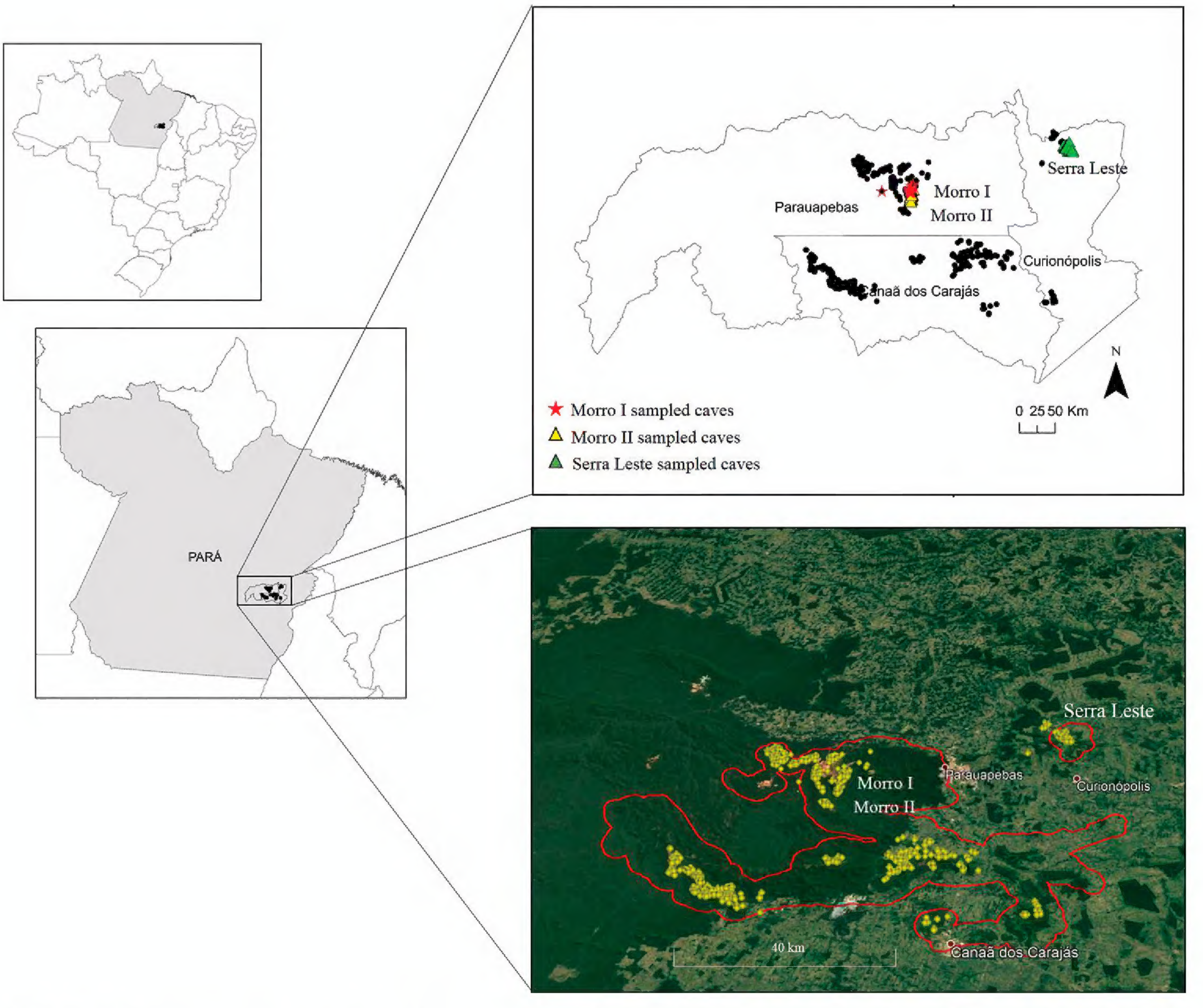

Figure 1. Ferruginous geosystem of Carajás, Pará, Brazil (limited by red lines), with iron ore caves (yellow dots) in the regions of Morro I, Morro II (Carajás National Forest) and Serra Leste. Limits of the ferruginous geosystem and the coordinates of the caves, available in https://institutopristino.org.br/atlas

40 km from Paraupebas and is a municipality known to house the Serra Pelada district, which was the world's leading area for open pit gold mining operations during 1980.

It has the largest iron ore mineral province on the planet, Serra dos Carajás, which has the highest concentration of ferruginous caves in Brazil, with approximately 20% of all caves registered in the country (Jaffé et al. 2016; Instituto Prístino 2020). The extraction of iron ore represents the main source of resources in the municipalities.

Both municipalities have a semi-humid tropical climate (Aw/As), with annual temperatures around 26 °C and high relative humidity; the rainy season occurs, from November to May, and the dry season, from June to October, with the rainfall index being around 2,000 mm annually (IBGE 2010).

Phlebotomine collections

Entomological captures were carried out in the years 2010, 2013, 2014 and 2015, through active samples with brushes, along the entire caves' extension, in dry and rainy periods from speleological activities carried out by independent consulting com-

panies, in the years, but using similar collection methods (Jaffé et al. 2016; Travellin et al. 2019). Not all sighted individuals were collected, since the authorization for collecting invertebrates in caves, issued by the Sistema de Autorização e Informação em Biodiversidade (SISBio) suggests employing a collection or capture effort that does not compromise the viability of populations in the taxonomic group of interest *in situ* condition (Normative Instruction N° 03, 01 September 2014). Not sampled sand flies were plotted on a schematic sketch of each cave and later counted from the schematic sketch and inserted in a spreadsheet of the database at Coleção de Invertebrados Subterrâneos de Lavras (ISLA). Then, the data of the total abundance of sand flies in the caves were extracted from the ISLA database. Such data refer to the counting of specimens in the field during the collections and were only used to identify greater or lesser probabilities of leishmaniasis transmission (considering the abundance as a *proxy* of transmission, probability), between areas and between seasons, especially in places with free access to residents and visitors (e.g. Serra Leste). All study material was found at the ISLA of the Center of Studies on Subterranean Biology, at the Federal University of Lavras (biologiasubterranea.com.br).

Preparation, assembly and identification of specimens

The collected specimens were sent to the Parasitology Laboratory of the Department of Biological Sciences at the Federal University of Jequitinhonha and Mucuri Valleys. The sandflies were prepared and assembled between a slide and coverslip, according to the Langeron technique (1949), modified, using Berlese's liquid to mount both sexes. The identification of the specimens was carried out according to the classification proposed by Galati (2003) and the abbreviation of generic names followed the work of Marcondes (2007). Some specimens with missing or damaged characters that impaired the identification at the specific level were considered only at the generic level (i.e., *Micropygomyia* spp.) or not identified.

Data analysis

The qualitative similarity was obtained by the Jaccard index and contrasted in a metric multidimensional scaling (MDS) with resampling using the Bootstrap method. Furthermore, the significant separation of species groups between the different sampling areas (Morro I, Morro II and Serra Leste) was evaluated by similarity analysis – ANOSIM (Clarke et al. 2014). To assess the mean dissimilarity between the sampling regions, SIMPER analysis (similarity percentage) was used. In addition, the percentage of species contribution to dissimilarity was evaluated (Clarke et al. 2014).

Differences in mean richness and abundance between areas and between dry and rainy periods were assessed using the Kruskal-Wallis test (Sprent and Smeeton 2000). To assess the eventual relationship between the sand fly's richness and the linear extension of the caves, a simple linear regression analysis was used. The normality of the data was tested using the Shapiro-Wilk test (Sokal and Rohlf 1995).

Results

In total, 306 iron ore caves (44 caves in Morro I, 115 in Morro II and 147 in Serra Leste) were inspected, and the phlebotomine fauna captured in 276 of these caves in 532 samplings (dry and rainy seasons) consisted of 8 genera and 17 species, namely: *Evandromyia carmelinoi* (Ryan, Fraiha, Lainson & Shaw, 1986), *Evandromyia monstruosa* (Floch & Abonnenc, 1944), *Evandromyia saulensis* (Floch & Abonnenc, 1944), *Evandromyia termitophila* (Martins, Falcão & Silva, 1964), *Lutzomyia longipalpis* (Lutz & Neiva, 1912), *Micropygomyia goiana* (Martins, Falcão & Silva), *Micropygomyia oswaldoi* (Mangabeira, 1942), *Micropygomyia peresi* (Mangabeira, 1942), *Micropygomyia pilosa* (Damasceno & Causey, 1944), *Nyssomyia umbratilis* (Ward & Fraiha, 1977), *Pintomyia* série *chagasi*, *Pintomyia gruta* (Ryan, 1986), *Pintomyia serrana* (Damasceno & Arouck, 1949), *Psathyromyia lutziana* (Costa Lima, 1932), *Sciopemyia sordellii* (Shannon & Del Ponte, 1927), *Trichophoromyia brachipyga* (Mangabeira, 1942), *Trichopygomyia dasypodogeton* (Castro, 1939) and *Micropygomyia* spp., totaling 802 specimens, being 369 males (46%) and 433 females (54%). The predominant species was *Sciopemyia sordellii* with 60.6% of the total of sand flies identified (Table 1). Specimens that had lost structures and could not be identified represented 15.21% of the total captured.

Similarity analysis (ANOSIM) showed a significant difference between the species composition of the Parauapebas and Curionópolis caves ($R = 0.06$ and $P < 0.05$) (Figure 2). The shade plot shows the species distribution in the Morro I, Morro II and

Table 1. Distribution of sand flies collected in iron ore caves in the State of Pará, Brazil, in the years 2010, 2013, 2014 and 2015.

Species	Curionópolis		Parauapebas		N	%
	♂	♀	♂	♀		
Evandromyia carmelinoi	2	1	0	0	3	0.38
Ev. monstruosa	0	1	0	1	2	0.24
Ev. saulensis	0	1	13	10	24	3.00
Ev. termitophila	0	2	0	0	2	0.24
Lutzomyia longipalpis	13	13	1	2	29	3.62
Micropygomyia goiana	0	0	0	3	3	0.38
Mi. oswaldoi	0	3	0	0	3	0.38
Mi. peresi	11	2	2	2	17	2.12
Mi. pilosa	2	1	1	1	5	0.63
Micropygomyia spp.	17	0	11	3	31	3.87
Nyssomyia umbratilis	0	0	1	1	2	0.24
Pintomyia série *chagasi*	0	0	0	6	6	0.75
Pi. gruta	7	9	25	22	63	7.86
Pi. serrana	0	0	0	1	1	0.12
Psathyromyia lutziana	0	0	0	1	1	0.12
Sciopemyia sordellii	46	64	184	192	486	60.60
Trichophoromyia brachipyga	0	0	1	0	1	0.12
Trichopygomyia dasypodogeton	0	0	1	0	1	0.12
Not identified	2	8	29	83	122	15.21
Sub-total	100	105	269	328	802	100
Total	205 (25.5%)		597 (74.5%)			

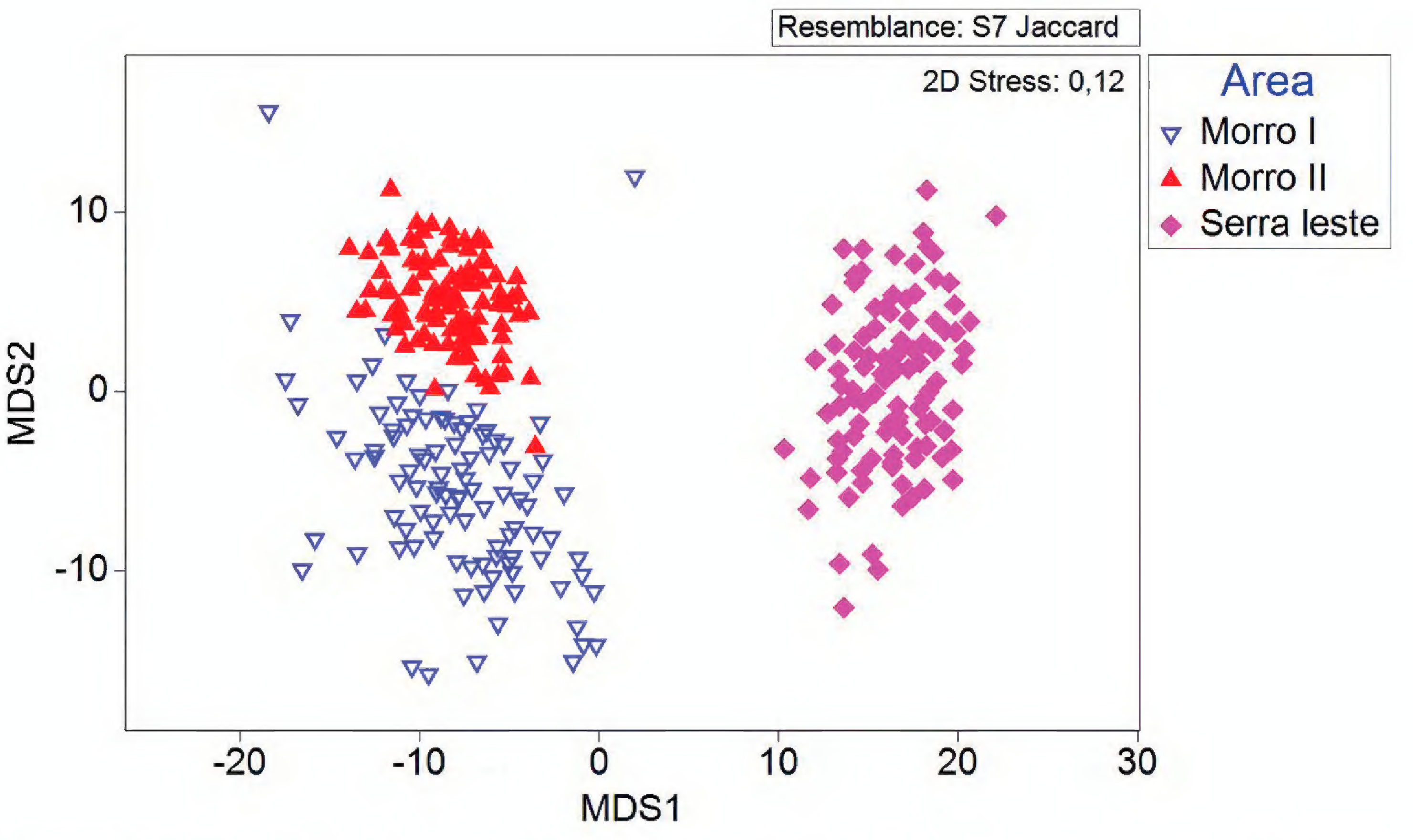

Figure 2. Metric multidimensional scaling (MDS) using bootstrap to show variations on sand flies species composition in iron ore caves at Parauapebas (Morro I, Morro II) and Curionópolis (Serra Leste) municipalities, Pará, Brazil.

Serra Leste caves, with the metric multidimensional scaling (MDS) showing the dispersion in the similarity of the fauna in the three sampled areas (Figures 2, 3).

The mean dissimilarity between the caves of Parauapebas and Curionópolis corresponded to 72.29, and the species responsible for the dissimilarity were *Sciopemyia sordellii* (77.26% contribution), *Pintomyia gruta* (12.01% contribution) and *Lutzomyia longipalpis* (7.92% contribution).

The mean richness was different between the two sampled areas (KW-H (1; 206) = 20.34; p <0.01), with a higher mean richness in the Parauapebas caves (mean = 2 spp., sd = 1) in relation to Curionópolis (mean = 1.44 spp., sd = 0.7). The mean richness was different between the regions of Morro II and Serra Leste (KW-H (2; 206) = 20.36; p <0.01), with a higher mean richness in the Morro I caves (mean = 2 spp., sd = 1.1) in relation to Morro II (mean = 1.98 spp., sd = 0.94) and Serra Leste (mean = 1.44 spp., sd = 0.7). The richness did not show any significant relation with the linear extension of the caves.

A total of 9,807 individuals were counted during the 532 samplings, 4,340 in the dry period and 5,467 in the rainy period. In the Morro I and II caves, 6,791 specimens (295 samples) were counted and 3,016 in Serra Leste (238 samples) (Suppl. material 1).

The mean abundance was different between the two sampled areas (KW-H (1; 532) = 28.37; p <0.01), with a higher mean abundance in the caves of Parauapebas (mean = 23.10 individuals, sd = 31.73) in relation to Curionópolis (mean = 13.60 individuals, sd = 29.40) (Figure 4A). The mean total abundance was different between the regions of Morro I, Morro II and Serra Leste (KW-H (2; 532) = 37.87; p <0.01), with a higher mean abundance in the Morro I caves (mean = 24.8 individuals, sd = 43.76)

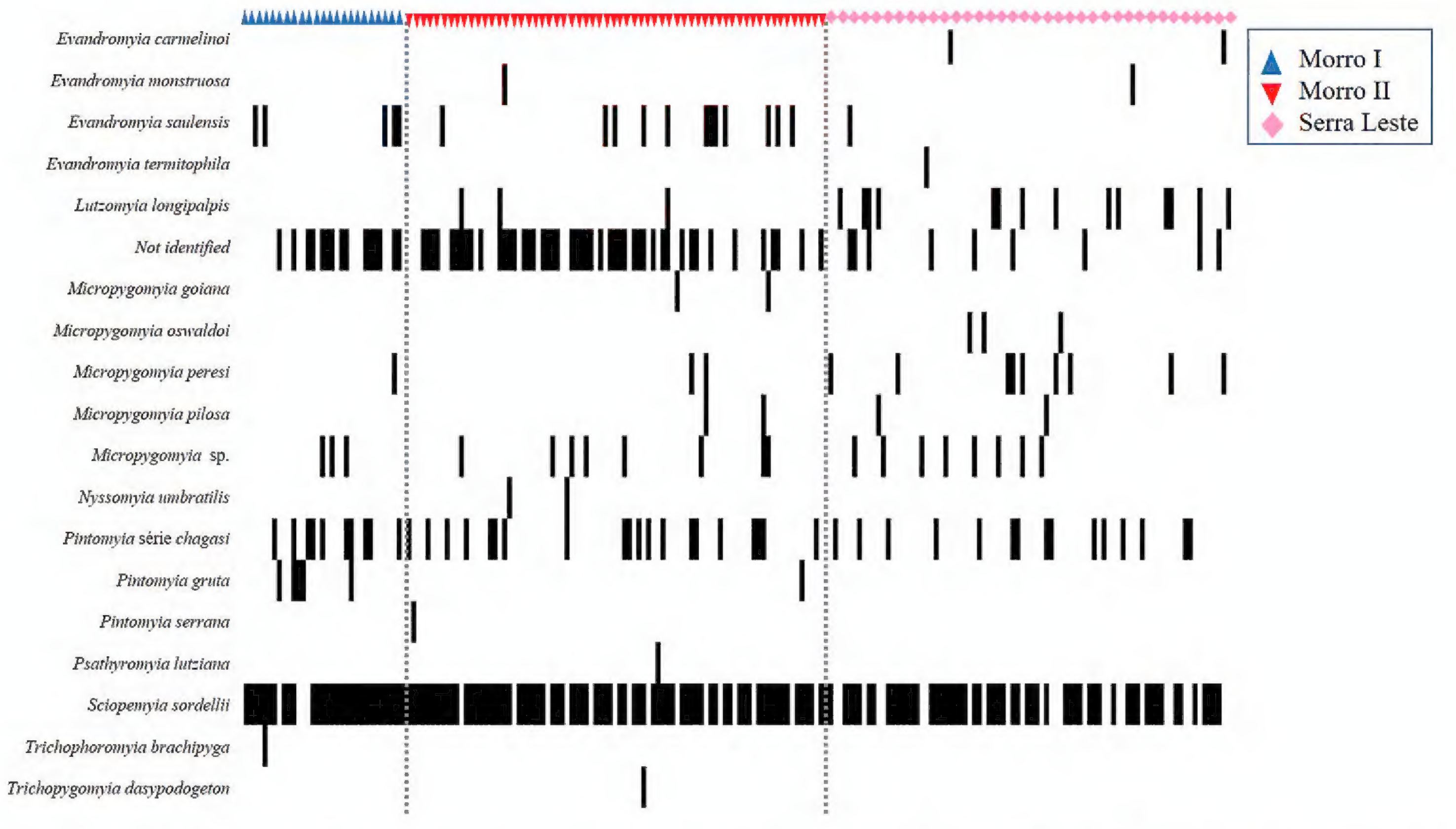

Figure 3. Distribution of species of sand flies collected in caves of Parauapebas (Morro I, Morro II) and Curionópolis (Serra Leste) municipalities, Pará, Brazil.

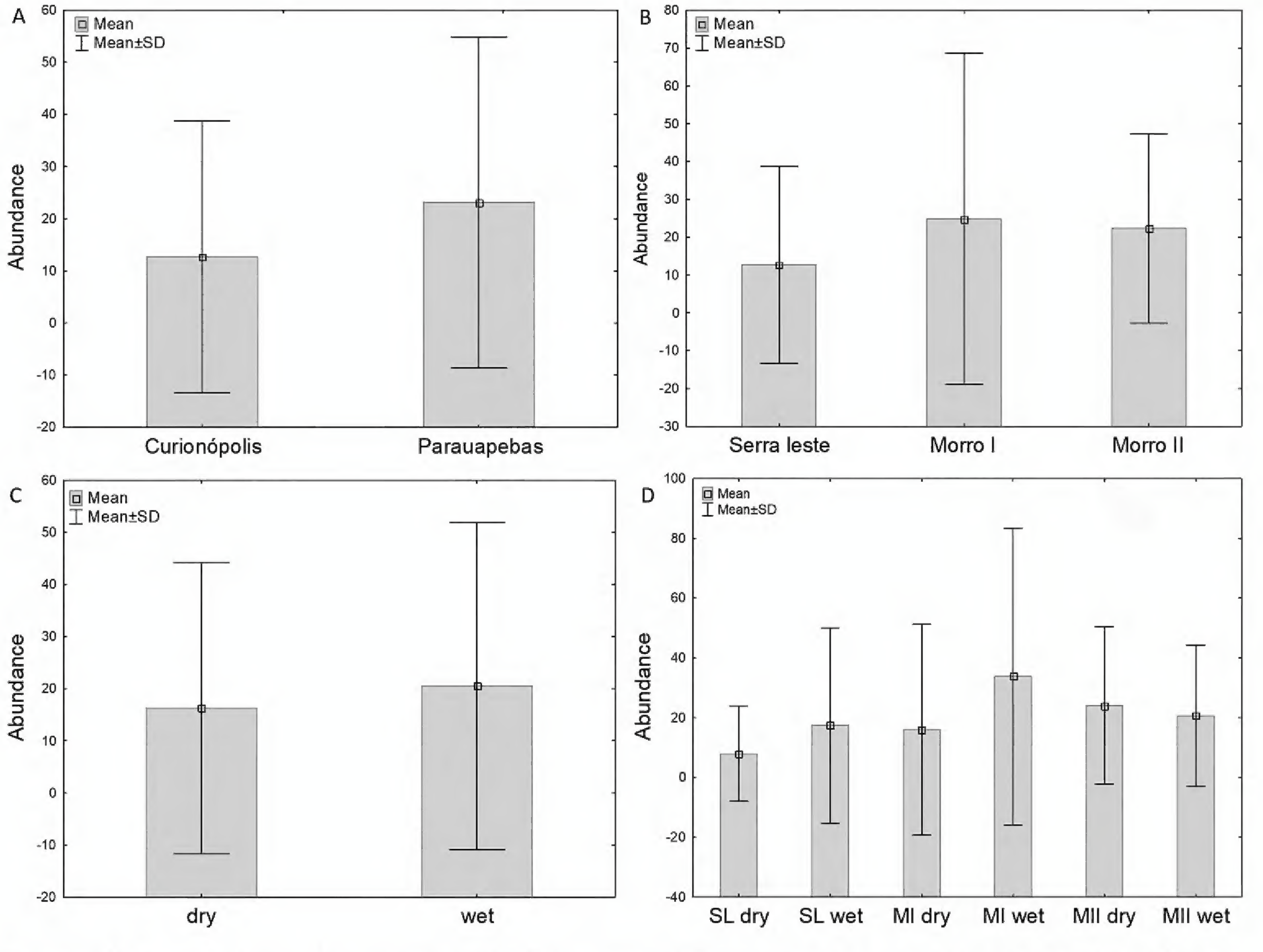

Figure 4. Distribution of abundance of sand flies counted in caves of Parauapebas and Curionópolis (**A**), in the regions of Morro I, Morro II and Serra Leste (**B**) in the dry and rainy periods of the year (**C, D**), Morro (MI), Morro II (MII) and Serra Leste (SL). Negative values do not represent negative abundance but the mean value subtracted from the standard deviation.

in relation to Morro II (mean = 22.3 individuals, sd = 24.98) and Serra Leste (mean = 13.60 individuals, sd = 29.39) (Figure 4B).

Considering all areas, the mean abundance was different between the dry and rainy periods (KW-H (1; 532) = 4.14; p <0.01), with a higher mean abundance in the rainy season (mean = 21 individuals, sd = 33.83) in relation to the dry (mean = 16 individuals, sd = 27.70) (Figure 4C). Considering each area independently, the mean total abundance was different between the dry and rainy periods only in Morro I (KW-H (1; 88) = 6.33; p <0.01), with a higher mean abundance in the rainy period (mean = 33, 79 individuals, sd = 49.7) in relation to the dry (mean = 15.97 individuals, sd = 35.2) (Figure 4D).

Discussion

Despite their restrictive traits, such as the scarcity of food resources, which could preclude the establishment of epigean species, caves can present a diverse and peculiar fauna. In this sense, inventories carried out in caves, particularly of sand flies, are extremely important, as they can contribute to the knowledge of the fauna of these insects, generating data of taxonomic, ecological and epidemiological importance (Pinto et al. 2012).

The use of light traps has been widely used in studies of sand flies in caves (Galati et al. 2010; Alves et al. 2011; Barata and Apolinário 2012; Carvalho et al. 2013). Such methodology is justified by the fact that it allows a longer time of exposure in the collections, characterizing the abundance more efficiently and preserving fragile morphological structures, essential for the identification of these insects at the specific level (head, wings, chest, legs, abdomen). However, the constant lighting can attract individuals who potentially would not be using the caves as a shelter or feeding place. Thus, the use of the manual collection is justified, with the use of brushes moistened with 70% alcohol when rapid assessments are intended and/or to minimize the attractiveness bias of light traps, especially in places closer to the cave entrances. However, care for specimens during collection and transport must be increased to avoid damaging morphological structures (Feitosa and Castellón 2004).

Sciopemyia sordellii was predominant (60.6% of the total number of specimens captured) and had a higher number of occurrences in the caves. Other authors have also recorded this species in caves (Galati et al. 1997; Carvalho et al. 2013), but also in other habitats, as in wild animal dens, tree trunks and tops, tabular roots, domestic animal attachments and in the human home (Aguiar and Medeiros 2003). Despite not being identified as a *Leishmania* vector, Guimarães et al. (2014) found DNA from this parasite in *Sc. sordellii* for the first time in Brazil. However, further studies are needed to clarify the role of this species in the transmission of the parasite, mainly due to its wide distribution and local abundance.

Pintomyia gruta represented 7.86% of the collected sand flies. This species is endemic to the Northeastern part of the country, and until a few years ago, its occurrence was restricted to the caves of Serra dos Carajás (Ryan 1986; Aguiar and Medeiros 2003). In 2015, specimens of *Pi. gruta* were captured in an area close to a hydroelectric

system in the state of Rondônia (Galardo et al. 2015), expanding knowledge about its occurrence and distribution. New records were also made in caves in Rondônia (Ogawa et al. 2016).

Among the most important findings of this work, the first records of *Pi. serrana*, *Tr. brachipyga*, *Tr. dasypodogeton* and *Ev. saulensis* in Brazilian caves must be highlighted. Despite the latter not be considered vector species of *Leishmania*, Araújo Pereira et al. (2017) and Avila et al. (2018) reported their infection in specimens collected in the state of Acre, highlighting the need to investigate the possible role of this species in the transmission of *Leishmania*.

Females of the *Mi. peresi* species, as well as *Ev. saulensis*, feed on ectothermic animals, such as reptiles and amphibians. Possibly, the registration of these species in caves is associated with the presence of reptiles and amphibians, which use this environment as a shelter (Matavelli et al. 2015). It is worth mentioning that the troglophilic habit of *Mi. peresi* has already been reported by Galati et al. (1997).

The species *Micropygomyia* spp. collected in the caves of the municipalities of Curionópolis and Parauapebas did not have their identification confirmed. It is important to highlight that it is probably a new species, which needs to be further studied. For this, new collections should be carried out in these places in search of new specimens for a future description of this species.

Two specimens of *Evandromyia termitophila* were reported in captures in ferruginous caves in Pará. Santos et al. (2011) captured this species close to a cave, but not inside. Therefore, this is not an unprecedented record of this species in Pará, but rather, the first notification of *Ev. termitophila* in caves in the state.

Considering the epidemiological importance of some species, we call attention to the record of *Ny. umbratilis*, which had also been recorded in caves in the State of Amazonas and Rondônia (Alves et al. 2011; Ogawa et al. 2016). *Nyssomyia umbratilis* is a vector for *Leishmania* (*Viannia*) *guyanensis* in Brazil (Lainson et al. 1979; Young and Duncan 1994; Souza et al. 2003; Feitosa and Castellon 2009). Other authors have found specimens of *Ny. umbratilis* infected also by *Leishmania braziliensis* (Arias and Freitas 1978).

Lutzomyia longipalpis species must be highlighted because it is the most important species in the epidemiological cycle of visceral leishmaniasis in Latin America (Brasil 2016). Infectious forms of *Leishmania infantum* have already been identified in this species by many authors (Lainson et al. 1987; Michalsky et al. 2011; Lidani et al. 2017). Due to its great ecological value and adaptive plasticity, it is currently considered an urbanized species, being captured mainly at home and in shelters for domestic animals (Barata et al. 2005; Dias et al. 2007; Michalsky et al. 2009; Chagas et al. 2016). However, the record of *Lu. longipalpis* in caves is still being documented (Galati et al. 2006; Galati et al. 2010; Carvalho et al. 2013; Campos 2017), revealing that some populations can still be found in wild and preserved places, such as some caves of this study.

The differences in composition and the lower richness and abundance found for the Serra Leste caves (Curionópolis), despite being weak may be related to the effect of environmental degradation observed in this region. Studies carried out outside cave

environments have shown a reduction ratio of sand flies in areas with capoeira vegetation, rural and urban areas concerning to forested areas (Araújo et al. 2000; Barros et al. 2000; Carvalho et al. 2000). Such reduction in richness and abundance and differences in species composition are probably because, in general, sand flies prefer shaded environments, with higher humidity and greater availability of organic matter (Aguiar and Medeiros 2003). However, some species may be more tolerant and occur in greater numbers in anthropized areas (Araújo et al. 2000; Carvalho et al. 2000).

In Brazil, a greater number of sand flies has been associated with the rainiest periods of the year (Marinho et al. 2008; Feitosa and Castellon 2009; Barata and Apolinário 2012) and this probably related to the greater availability of blood sources, with a higher rate of oviposition and, thus, providing a greater occurrence of larvae and, later, adults. The higher abundance of these insects in the rainy season, with the presence of proven vectors, suggests a greater risk of leishmaniasis transmission at this time.

The phlebotomine fauna was shown to be rich and abundant in the sampled caves, however, environmental degradation seems to be the main factor in producing changes in composition and richness, especially in the Serra Leste region. In addition, the large number of species in the caves of Morro I and II, in comparison to Serra Leste, reinforces the importance of these places as a shelter for sand flies in degraded areas.

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Supplementary material 1

Table S1

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